

Program Notice

FGIS-PN-06-02

03-27-06

PERFORMANCE EVALUATION CRITERIA FOR DEOXYNIVALENOL (DON) TEST KITS

1. PURPOSE

This Program Notice informs interested parties, particularly deoxynivalenol (also known as vomitoxin) test kit manufacturers, that the Federal Grain Inspection Service (FGIS) of the Grain Inspection, Packers and Stockyards Administration (GIPSA) has issued an updated criteria document for performance evaluations of quantitative vomitoxin test kits submitted for GIPSA approval/certification. Current Certificates of Conformance based on the old performance criteria, will expire on September 30, 2007.

2. EFFECTIVE DATE

This action is effective upon receipt.

3. BACKGROUND

Since 1990, GIPSA's Technical Services Division (TSD) has provided performance evaluations of mycotoxin test kits for private corporations seeking GIPSA approval for their kits to be used in the Official Inspection System. The "Design Criteria and Test Performance Specifications for Quantitative Deoxynivalenol (DON) Test Kits" document is used by TSD to determine if a test kit meets the criteria for quantitative determination of vomitoxin in grains, and processed-grain products for official testing purposes. The original criteria required test kits to be evaluated for accuracy and precision using fortified vomitoxin-free wheat samples and naturally-contaminated wheat samples. After careful review of the original criteria document and evaluation of numerous test kits, GIPSA has determined that test kits often perform differently when analyzing naturally-contaminated samples as compared to analyzing fortified samples. In addition, there is a need for increased accuracy at lower vomitoxin levels.

Considering these factors, the performance criteria document has been rewritten with a focus on performance requirements at lower levels and on naturally-contaminated wheat samples, which reflects the conditions under which the kits are used. The updated criteria document (attached) will allow manufacturers to develop kits that perform accurately on these "real world" samples, thereby improving service to the grain industry.

4. QUESTIONS

Direct questions concerning test performance specifications for quantitative vomitoxin test kits to Lynn Polston, TSD, at (816) 891-0444; or e-mail at Lynn.A.Polston@usda.gov.

/s/ Steven N. Tanner

Steven N. Tanner, Director
Technical Services Division

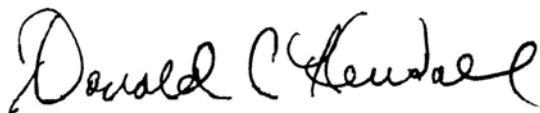
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DESIGN CRITERIA AND TEST PERFORMANCE SPECIFICATIONS FOR QUANTITATIVE DEOXYNIVALENOL (DON) TEST KITS

Prepared by
U.S. Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Technical Services Division



Approved By: _____ Date: March 6, 2006
Group Leader, Test Kit Evaluation Program



Approved By: _____ Date: March 6, 2006
Deputy Director, TSD

APPROVAL FACTORS

Each offeror must provide documentation to show that their test kit is capable of meeting all of the performance standards for quantitative test kits listed below. In addition, the Grain Inspection, Packers and Stockyards Administration (GIPSA) will verify that the test kit meets these performance standards.

Definitions

Deoxynivalenol (DON) - 3, 7, 15-trihydroxy-12,13-epoxytrichothec-9-en-8-one, also known as vomitoxin.

Malted Barley - Brewers or distillers malt produced by steeping and germinating barley at moisture levels ranging from 43% to 49% and high temperature (71 - 82° C) drying to approximately 4% moisture or low temperature (48 - 60° C) drying to approximately 5% to 7% moisture.

Fortified Samples - Samples containing a known, added amount of deoxynivalenol.

Test Kit - A commercially packaged system of the principal or key components of an analytical method used to determine the presence of a specific analyte(s) in a given matrix(es). Test kits include directions for their use and are self-contained, complete analytical systems, but they may require supporting supplies and equipment. The key components frequently represent proprietary elements or reagents that may be readily prepared only by the producer of the kit.

Performance Standards

Good Laboratory Practices. Good laboratory procedures must be followed when conducting experiments to acquire data to submit in support of specific test kit performance. The same person performing the analyses must not prepare the fortified samples. All samples must be labeled with a code and fully randomized so that the analyst conducting the test cannot know the level of analyte.

The deoxynivalenol used to fortify samples shall be well characterized and of known purity. A statement of analysis from the supplier shall be submitted with the data. Concentrations of the deoxynivalenol standard must be confirmed by UV spectrophotometry as specified in GIPSA's Deoxynivalenol Reference Method. A copy of this document may be obtained from GIPSA upon request.

A validated DON method must be used for analyzing the samples to be fortified with DON, including each commodity, and for analyzing the samples naturally contaminated with DON. A copy of the completed method validation report or literature reference to a published report shall be supplied by the applicant. The GIPSA Deoxynivalenol Reference Method will be used in the final GIPSA Performance Verification. A copy of this reference method may be obtained from GIPSA upon request.

1. Time required for completion of an analysis.

A test kit must be capable of quantifying deoxynivalenol residues in a single, pre-ground sample in less than 30 minutes. For example, a test that would require two hours for the extraction and simultaneous analysis of four samples would not be acceptable. If the standards supplied with the test kit do not cover the range specified, the total time for completing a sample analysis, including dilutions, must meet the thirty-minute requirement.

2. The limit of detection (LOD) of offered test kits should be less than or equal to 0.25 ppm.

The LOD is defined as follows: $LOD = (\text{mean}) + 2 * (SD)$. The mean is determined from readings of 10 different extracts of a deoxynivalenol-free sample SD is the standard deviation of those 10 readings. Offerors must provide the LOD for their test kits. Test kits not meeting the LOD requirement for wheat will not be considered for approval. **LOD determinations shall be made on each commodity for which the test kit is seeking approval.**

3. Comparative accuracy of test kits on wheat samples naturally contaminated with deoxynivalenol.

Four wheat samples naturally contaminated with deoxynivalenol at 0.50, 1.00, 2.00, and 5.00 ppm ($\pm 15\%$) shall be ground so that 95% of the material passes through a 20-mesh sieve. The wheat sample at **each** concentration level will be divided into forty-two sets of 50-g subportions using a method that will ensure that resultant subportions are well mixed and homogeneous. These subportions will be placed in specimen containers, sealed, and stored at 5° C until needed.

Three analysts shall each extract seven subportions of the sample at each level according to test kit instructions. One analysis of each extract shall be made by each of three analysts. Each operator shall use a separate kit for his or her analyses. No sharing of test kits will be allowed. In addition, twenty-one subportions of this sample will be extracted and analyzed by HPLC in three separate batches (seven samples per batch) using the validated Deoxynivalenol Reference Method.

At least 95% of the individual values at each concentration must be within the range specified in Table 1.

Table 1. Acceptable Limits

Concentration (ppm)	Maximum RSD _i (%)	Standard Deviation	Range* (ppm)
0.50	25	0.125	0.25 – 0.75
1.00	20	0.20	0.60 – 1.40
2.00	15	0.40	1.40 – 2.60
5.00	10	0.50	4.00 – 6.00

* For naturally contaminated samples, this range will be recalculated using the actual mean and the maximum RSD values at each concentration as listed. The mean will be determined by using the 21 HPLC values obtained using the validated Deoxynivalenol Reference Method.

4. Suggested additional commodities: barley, malted barley, oats, and corn.

For a test kit to be approved for additional commodities, offerors must submit procedures and documented data showing accuracy of tests for each commodity. Uncontaminated samples of each commodity must be ground with a mill so that over 95% of the material passes through a 20-mesh sieve. Ground portions must be divided into 50-g subportions using a method that will ensure that resultant subportions are well mixed and homogenous. Specific information on the equipment and procedures that were used in the grinding, mixing, and sieving processes must be provided.

A minimum of five fortified samples for each matrix with 0.50 ppm deoxynivalenol and minimum of five fortified samples for each matrix with 2.00 ppm deoxynivalenol concentrations should be prepared using a confirmed standard (as described in Good Laboratory Practices). Samples should be left open in an exhaust hood for 30 minutes after fortification and stored at 5° C until analyzed.

One operator shall extract samples according to test kit instructions. For each and every matrix, a single analyst shall perform one analysis of each extract using the appropriate test kit materials including needed equipment not contained in the test kit. All analyses shall be conducted at room temperature. Controls shall be used with each individual analysis if required by the instructions for the test kit.

At least 95% of the individual values at each concentration must be within the range specified in Table 1.

5. Avoidance of toxic or hazardous substances.

Testing shall not expose employees to toxic or hazardous substances higher than OSHA Standards (29 CFR) or require special waste disposal. Items that might require special waste disposal include all radioactive materials, materials containing concentrations of substances that exceed the Environmental Protection Agency (EPA) Maximum Concentrations of Contaminants for the Toxicity Characteristic found in 40 CFR, and any materials having the EPA Hazardous Waste Number D003 as defined in 40 CFR. Material Safety Data Sheets shall be provided for each test kit. Each offeror must provide information on waste disposal that meets EPA requirements for all chemicals used in their test kit. No test kit will be considered for approval that cannot meet the above criteria.

6. Sensitivity to Electromagnetic Fields

Electronic equipment offered with test kits shall be insensitive to electromagnetic fields of intensity of 5.0 volts/meter over the frequency range of 500 KHz to 1 GHz and less than or equal to 1.0 volt/meter over a frequency range of 1.1 GHz to 15 GHz. Offerors shall provide a statement of certification from an accredited laboratory to this effect in their proposal. Laboratories shall be accredited under the National Volunteer Laboratory Accreditation Program

operated by the National Institute of Standards and Technology, Gaithersburg, Maryland. Any electronic equipment not meeting this requirement will not be considered for approval.

7. Temperature sensitivity.

One naturally contaminated wheat sample at 1 ppm ($\pm 15\%$) DON concentration shall be prepared by grinding so that over 95% of the material passes through a 20-mesh sieve. The wheat sample shall be divided into seven 50-g subportions using a method that will ensure that resultant subportions are well mixed and homogenous. These sub portions will be placed in specimen containers, sealed, and stored at 5° C until needed.

One operator shall extract samples at room temperature according to test kit instructions. Extracted samples must be protected from light during this test.

Place extracts and all equipment needed to conduct an analysis in an environmental chamber set at 24° C. Allow to equilibrate for a period of one hour. Perform assays using manufacturer's recommended procedure.

Adjust room temperature to 18° C and allow extracts and equipment to equilibrate for one hour. Perform assays using manufacturer's recommended procedure.

Readjust the temperature of the environmental chamber to 30° C and allow extracts and equipment to equilibrate for one hour. Perform assays using manufacturer's recommended procedure.

At least 95% of the individual values must be within the applicable range specified in Table 1.

8. Stability.

Offerors must provide data on the stability of their materials and the reagents used in their tests under label-recommended storage times and conditions that support claimed expiration dating and lot-to-lot variability. This data should confirm conformance to all applicable criteria in this document.

9. GIPSA Performance Verification on Naturally Contaminated Samples.

Offerors will be required to supply all test kits, equipment, and supplies needed to complete the verification tests described below.

Three wheat samples naturally contaminated with deoxynivalenol concentrations of approximately 0.5, 2.0 and 5.0 ppm ($\pm 15\%$) will be ground to meet the sieve specifications given in Section 3. The ground sample will be mixed and divided into forty-two sets of 50-g subportions. These sub portions will be placed in specimen containers, sealed, and stored at 5° C until needed.

Three analysts shall each extract seven sub portions at each level according to test kit instructions. One analysis of each extract will be made by each of the three operators. In

addition, twenty-one subportions of the sample at each level, will be extracted and analyzed by HPLC in three separate batches (seven samples per batch) using the GIPSA Deoxynivalenol Reference Method.

For GIPSA approval, at least 95% of the individual values at each concentration must be within the range as specified in Table 1.

Sample Extraction and Analyses. Sample extraction and analyses will be done according to the offeror's directions. All analyses will be conducted at room temperature (approximately 21° C). If a control is required for the test, then a control will be used with each individual sample analysis.

Operators and Training. Three operators will conduct all analyses. All operators shall receive up to two days of training in the use of each test kit. The training is to be provided by the offeror. In addition, each operator shall receive detailed, step-by-step, written directions, prepared by the offeror, on the use of each test kit.

Outliers. If an observation is a suspected outlier, the available documentation will be reviewed to identify the source of the error and make corrections where possible. Identifiable errors (not the fault of the test method) that cannot be corrected will result in the observation being discarded. Suspected outliers with unidentifiable causes will be tested using methods described in the AOAC manual, "Use of Statistics to Develop and Evaluate Analytical Methods," by G. T. Wernimont (p. 96 and Table A-8). No more than one outlier per test kit will be discarded.

PROTOCOL & NOTIFICATION AGREEMENT STATEMENT

This is to certify that I am an Official Representative of _____ ,
that I fully understand the conditions which GIPSA will use to determine if our quantitative
deoxynivalenol test kit marketed under the trade name

_____ will be given a
Certificate of Conformance for use in the official inspection system. The Certificate of
Conformance will be valid for three years from the date it is issued.

GIPSA monitors the performance of all approved test kits, and reserves the right to conduct a
performance verification of the test kit based on data obtained through GIPSA's quality
assurance programs. I agree to pay the standard GIPSA hourly rate for all service hours required
for training and evaluation of the test kit. I understand that if the kit fails to meet the criteria set
forth in the performance specification document, the Certificate of Conformance will be revoked
immediately. I further understand that the Certificate of Conformance will expire after three
years and the kit must be completely re-evaluated to renew the Certificate of Conformance. I
accept these conditions and I also agree to abide by the Manufacturer's Notification
Responsibilities provided in this document.

Name

Date

Title

Manufacturer's Notification Responsibilities

Manufacturers with test kits on the list of equipment approved for official inspection must notify the Director, Technical Services Division, USDA-GIPSA Technical Center, 10383 N. Ambassador Drive, Kansas City, Missouri 64153-1394, in writing when any changes or alterations are made to the approved test kit, to any reagents, to the kit shelf life, or to equipment used in the test kit, or to any part of the analytical method. Any alterations to a test kit will require a full GIPSA performance verification. Failure to notify GIPSA of these changes will serve as grounds for immediate withdrawal of the test kit from the approved list. Changes in the packaging, brochure, or other marketing information about the test kit are exempt from this requirement.